REMARKS

This is in response to the Office Action dated March 25, 2008. Claims 17-37 were acted upon by the Examiner. Claim 17 has been amended. Claim 38 has been added. No claims have been canceled. Accordingly, claims 17-38 are presented for examination.

Amendments to the Claims

Claim 17 is amended in several respects for clarity. Each of the amendments is supported by the specification as discussed below.

With respect to step (a)(i), the term capture probe has been replaced with hybridization probe. As the specification makes clear immobilized hybridization probes are sometimes referred to as capture probes. See specification at page 7, line 6. Accordingly, because it is clear from step (a) that the probe is immobilized on the surface of a microcarrier, for clarity the term capture probe has been replaced with hybridization probe. For additional clarity we have also made clear that the probe is directed to one of said multiplicity of sequences and that the hybridization of the probe to one of said at least one sequence can be detected. As is explained on page 7, while molecular beacon probes are the most preferred embodiment of immobilized target hybridization probes, this invention is generally applicable to methods where hybridization of the probe to its target can be detected. See specification at page 7, lines 4-25. This is also consistent with step (c).

With respect to step (b) of claim 17, for clarity, the step is amended regarding the term dstributed array by adding the definition appearing at page 2, line 13, namely, an array where the location of the microcarriers is not used to identify said at least one sequence.

With respect to step (d) of claim 17, the step is amended to recite that decoding includes changing the environmental condition to said detectably different levels of the condition. As the claim now recites the action taken during decoding, that is, changing the condition, there is no need to describe the condition as controllable ,so that word is deleted from claim 17. The

environmental condition most discussed in this application is temperature, and changing it through three disruption levels (for oligonucleotide stems that means disrupting by melting the stem) is described at page 7, lines 27-30. FIGS. 6A-6C, discussed at page 12, line 11 to page 13, line14, show disruption of different hairpins occurring at different levels as the condition is changed, where the condition being changed is temperature, formamide concentration, and salt (guanidine thiocyanate) concentration. As explained at page 13, lines 2-14, the condition may be temperature, salt ions, concentration of co-solvents such as ethanol and formamide, changing concentration of urea and chaotropic agents, changing pH, and changing denaturants for antibody or protein affinity pairs.

New claim 38 has been added which depends from claim 17. Support for claim 38 can be found, for example, at page 14, lines 9-12 of the specification. No issue of new matter is seen.

Summary of the Presently Claimed Invention

The presently claimed invention is directed to a hybridization assay for at least one of a multiplicity of nucleic acid sequences in an analyte that uses a unique and nonobvious coding scheme. As amended, claim 17 (the only independent claim pending) comprises the steps of (a) contacting the analyte with a mixture of encoded microcarriers, (b) forming a distributed array of said microcarriers; (c) determining which microcarriers have hybridization probes hybridized to said at least one nucleic acid sequence of said analyte; and (d) optically decoding the microcarriers having hybridized hybridization probes to identify said at least one nucleic acid sequence.

The hybridization assay of the presently claimed invention uses unique and non-obvious microcarriers that have immobilized on their surface (i) a hybridization probe, and (ii) a coding scheme comprising a plurality of signaling hairpins. The hybridization probe functions to ***hybridize*** to the target (i.e., hybridize to one of said multiplicity of sequences). This hybridization is detectable to be able to determine in step (c) which microcarriers have hybridization probes

hybridized to their target. The coding scheme then allows for the optical decoding of the microcarrier to identify the at least one nucleic acid sequence.

As recited in the claim, the signaling hairpins comprise quenched, fluorophore-labeled hairpin molecules which are disruptable by a physical or chemical change in their environment. The method includes forming a distributed array of the microcarriers, some of which now contain one hybridized target or another, such that each can be addressed individually. In that state, one identifies which of the microcarriers have a target hybridized to them. Because the array is a distributed array, ***location of a microcarrier in the array does not identify which hybridization probe it carries***. Rather, decoding is done by altering an environmental condition, for example temperature, to disrupt the signaling hairpins on the bead and thereby generate fluorescence signals. Claim 17 requires the use of at least two levels of the condition, claim 19 requires three, and claim 20 requires 3-8.

The coding scheme of the present invention involves the combination of signals of different colors generated at different levels of a condition, for example different temperatures. By way of example, if a signal, say red, rises to an intensity of 1 at 58 °C (first condition level), and jumps to an intensity of 2 at 72 °C (second condition level), one knows that the microbead being addressed has a combination of two red-signaling hairpins, one having each of those melting temperatures. If a red signal arises to an intensity of 1 at 58 °C (first condition level), but at 72 °C (second condition level) the red signal jumps to an intensity of 2 and a green signal appears as well, one knows that the microbead being addressed has a combination of two red-signaling hairpins having each of those melting temperatures plus a green-signaling hairpin having a melting temperature of 72 °C. In either case one then only determines which hybridization probe was immobilized on the microbead so encoded, and the hybridized target is identified. The code embodiment discussed in the first paragraph on page 7 utilizes three condition levels and five fluorophores, yielding 32,768 code elements by which 32,768 different

hybridization probes (in other words, 32,768 different bead types) can be identified for 32,768 different target sequences.

Discussion of Examiner s Priority Date

The Examiner asserts that the application will now be entitled to a new priority date of January 2, 2008, the date of applicants RCE and Reply, because applicants added new matter to the specification. This is improper. First, as explained in applicants prior Reply, the two paragraphs are supported by claims 1, 4, 7, and 8 as originally filed. In establishing a disclosure, applicant may rely not only on the description and drawing as filed but also on the original claims if their content justifies it. M.P.E.P. § 608.01(I). In such a case, applicants are entitled to amend the description to accord with the originally claimed subject matter. *Id.* That is what applicants did here. Second, even if the amendment are new matter ' and it is not ' the proper remedy is to object to the amendment as adding new matter. M.P.E.P. § 608.04.

Discussion of Examiner s Claim Interpretation

The Examiner interprets capture probe to mean any probe. Applicants have clarified the claimed invention by replacing the term capture probe with hybridization probe . Accordingly, the hybridization probe of the claimed invention hybridizes to one of said multiplicity of sequences. As per claim 17, the hybridization probe is also immobilized on a microcarrier. Hybridization probes also encompass labeled and unlabeled oligonucleotides that may be linear or may have a special structure, for example, molecular beacon probes. See specification at page 7, lines 9-15.

The Examiner interprets controllable broadly, as the term controllable is not defined per se in the specification. The Examiner interprets controllable to mean alteration of some underlying conditions. To improve clarity, applicants have amended claim 17 to remove the term controllable. In the method of claim 17, with respect to the signaling hairpins, a change is made

to a physical or chemical condition of their environment, and that change causes a structural change, namely, a disruption of the hairpin shape of the signaling hairpins.

The Examiner interprets a planar array to be any array. A planar array is an array on a planar solid surface (page 2, lines 6-7) rather than a three-dimensional array. Claim 17 recites that the microcarriers, after being mixed with the analyte, are formed into a distributed array in which location does not serve as an identifier as recited in amended claim 17.

Discussion of Section 103 Rejections

The Board of Patent Appeals and Interferences recently articulated the requirements for a *prima facie* case of obviousness in view of the Supreme Court *KSR* decision. The Board makes clear that an Examiner still has the burden of showing how the prior art references teach or suggest ***all*** the claim limitations.

> When determining whether a claim is obvious, an examiner must make a searching comparison of the claimed invention ' *including all its limitations* ' with the teaching of the prior art. *In re Ochiai*, 71 F.3d 1565, 1572 (Fed. Cir. 1995) (emphasis added). Thus, obviousnes requires a suggestion of all limitations in a claim. *CFMT, Inc. v. Yieldup Intern. Corp.*, 349 F.3d 1333, 1342 (Fed. Cir. 2003) (*citing In re Royka*, 490 F.2d 981, 985 (CCPA 1974)). Moreover, as the Supreme Court recently stated, *there must be some articulated reasoning* with some rational underpinning to support the legal conclusion of obviousness. *KSR Int l v. Teleflex Inc*, 127 S. Ct. 1727, 1741 (2007) (quoting In re Kahn, 441 F.3d 977, 988 (Fed. Cir. 2006) (emphasis added)).

Ex parte Wada and Murphy, Appeal No. 2007-3733 (BPAI January 14, 2008) (emphasis in original). Here, the references, individually or combined, do not teach or suggest all the claim limitations.

Claims 17-22, 24-31, and 33-38

Claims 17-22, 24-31, and 33-37 are rejected under 35 U.S.C. §103(a) as being unpatentable over Stanton et al. (U.S. Patent No. 6,680,377) in view of Liu et al. (Analytical

Biochemistry 283:56-63 (2000)); Lizardi et al. (U.S. Patent No. 5,312,728) and Chee et al. (WO 01/46675).

Stanton et al. is directed to aptamer beacons for simultaneously detecting the presence and quantity of one or more different compounds in a sample. An aptamer is an oligonucleotide that binds to a protein, small organic molecule, or inorganic molecule (col. 1, lines 59-61); in other words, to a non-nucleic acid target molecule (col. 2, lines 39-40). Stanton et al. discloses aptamers that are hairpin-shaped probes that signal when they bind to non-nucleic acid target molecules. Like molecular beacon probes that hybridize to nucleic acid sequences, aptamer beacons may be labeled with a fluorophore and a quencher such that when their binding to a target causes a conformational change, a fluorescent signal results. See Figure 3 Methods of use include immobilizing different aptamer beacons in a predetermined array on a solid support (col. 4, lines 9-11). Thus, the support may be planar or even linear, but it is the opposite of a distributed array, because location identifies which aptamer beacon is in a particular spot. Aptamer beacons can be applied to discrete locations on glass slides by pre-making the aptamer beacons and using a robotic micropipeter to apply them as microdrops to a functionalized slide surface (col. 11, lines 5-29) or by manufacturing aptamer beacons in situ using photoresist masking methods (col.11, line 66-col. 12, line 10).

Liu et al. discloses optical fibers on which are immobilized TMR-labeled molecular beacon probes (hairpin-shaped hybridization probes complementary to target) using biotin and streptavadin to immobilize the probes. Thus, the molecular beacon probes that find their target serve both as target-binding probes and as capture probes. In that regard they are like the immobilized hybridization probes of claim 17. Liu et al. reports that fluorescent signals caused by hybridization of the probes to targets could be detected with an inverted fluorescence microscope. Using a bundle of four fibers, two containing a first molecular beacon probe and two containing a second molecular beacon probe, signal from exposure to either or both of two targets could be detected by this method. As shown in FIG. 4 on page 61, each fiber in the

bundle was identified by location, so that signal could be attributed to the hybridizing molecular beacon(s) by location.

Lizardi et al. discloses a large hairpin-shaped hybridization probe called a molecular switch. The molecular switch is an unlabeled probe. When it hybridizes to a rare target and opens, one of the open arms, for example, can serve as a hybridization site for a DNA sequence required for a signal-generating reaction. See col. 5, lines 39-57 and Figures 1 and 2. The signal-generating reaction is preferably an amplification reaction that produces sufficient amount of an amplicon to be detected by known methods. See col. 8, lines 22-25. In one example in Lizardi et al. prior to amplification, hybridized (open) probes are separated from unbound (closed) probes through the use of separate capture probes that immobilize hybridization probe/target complexes to a solid surface, which is washed to remove unhybridized molecular switch probes. See col. 9, lines 64-66. Arrays are not seen to be a topic of Lizardi et al., nor are hybridization probes that are themselves immobilized on a solid surface, as claim 17 requires.

Chee et al. discloses systems of many hybridization probes, each different probe being immobilized on a microbead. Chee et al. refers to such target-binding probes as bioactive agents. The object of Chee et al. is to avoid having to insert optical signatures into the beads (use of many different hybridization probes requires the manufacture of many different beads with unique optical signatures embedded in them) as an earlier method required. Chee et al. presents as a solution the immobilization on the beads of a plurality of different identifier binding ligands (IBL s) from a panel of IBL s, which may be a panel of oligonucleotides. To decode a bead, that is, to determine which bioactive agent (target-hybridizing probe) it contains, the bead may be exposed to a solution of detectable decoder binding ligands (DBL s), which may be fluorophore labeled oligonucleotides that are complementary to the panel of IBL s. Detecting which DBL s bind to the bead by hybridizing to the bead s IBL s, reveals the bead s code and thereby identifies which target-binding hybridization probe (bioactive agent) the bead carries. Chee et al. also refers to oligonucleotide IBL s as identifier probes and to DBL s as decoder probes.

The DBL s may be arranged in a pattern of wells as a positional array, in which case the DBL s need not be labeled, or the DBL s may be labeled and free in solution, in which case beads can be formed into a distributed array for decoding which combination of DBL s hybridized to a particular bead.

As described in detail below, the cited references alone or in combination fail to teach or suggest each of the recitations of claim 17 (from which all the rejected claims depend).

Stanton et al.

First, Stanton et al. fails to teach or suggest a hybridization assay. Hybridization is the formation of double-stranded helical segments by the bonding of single-stranded nucleic acid strands (see, e.g., Stenesh, J., DICTIONARY OF BIOCHEMISTRY, John Wiley & Sons, Inc. (New York) 1975). In contrast, Stanton et al. teaches assays for non-nucleic acid molecule targets, which by definition are therefore not hybridization assays (col. 2, lines 39-40).

Second, Stanton et al. fails to teach or suggest encoded microcarriers. Stanton et al. teaches the use of aptamers bound to a solid support in a *predetermined* array to identify the target by location (col. 4, lines 9-11). Moreover, claim 17 recites a coding scheme comprising immobilized signaling hairpins that are not hybridization probes (formerly, that are not capture probes) for the at least one nucleic acid target. Contrary to the Examiner s assertion on page 5 of the Action, Stanton et al. s aptamer beacons are indeed capture probes (as well as labeled reporter probes, because, when bound to a solid support, they function to immobilize their non-nucleic acid target molecules to the beads (the functional definition of a capture probe, as the Examiner states at page 11 of the Action)). Stanton et al. s aptamer beacons are not part of any coding scheme, however, as Stanton et al. teaches use of a traditional positional array to identify which aptamer beacon, has bound a target molecule. Stanton et al. does not teach or suggest the use of other aptamer beacons *that do not bind to a target* and that could function as a signaling hairpins as recited in claim 17.

Moreover, Stanton et al. does not teach coding by the environmental condition of concentration. Rather, Stanton et al. teaches that the brightness of an array spot is dependent, not on an environmental condition change, but rather on the concentration of target that one happens to encounter in a particular sample (col. 5, lines 61-67). In Example 2, at cols. 23-28, Stanton et al. teaches an assay for thrombin. Three aptamer beacons that were labeled with fluorescein/DABCYL were tested for their ability to bind thrombin, the target, in different buffers at varying thrombin (target) concentration and to signal upon binding. Tests were performed to ensure that the signals detected were not simply due to a change in the chemical environment (col. 26, lines 48-50). The aptamer beacons opened when contacted with thrombin, as detector probes are meant to do. Similarly, Stanton et al. s teaching in column 4 that one aptamer beacon can be selected to bind to a first enantiomer (optical isomer) of a target and another aptamer beacon can be selected to bind to a second enantiomer of the target is not coding, but rather multiplexing of a plurality of hybridization/capture probes on the same or different beads. Using different fluorophores for different aptamer beacons, for which the Examiner cites column 15, lines 20-39, is also just multiplexing of up to six or eight different probes, without any coding scheme to identify beads that cannot be differentiated by their differently colored probes. Stanton et al. contains no teaching of some aptamer beacons that open at a first thrombin concentration and other aptamer beacons that open only at a second thrombin concentration. Accordingly, Stanton et al. steaching is in no way analogous to the teaching of the instant application of a plurality of signaling hairpins whose opening is made to occur, not by contact with target, but rather by an environmental change such as formamide concentration, some opening at a first concentration and others opening only at a second concentration.

Third, Stanton et al. does not teach or suggest forming a distributed array of microcarriers (or even a distributed array of aptamer beacons) wherein location of the microcarriers (or the beacons) in the distributed array ***is not used to identify the target.*** Rather, as mentioned above,

Stanton et al. teaches forming standard positional planar arrays (e.g., predetermined arrays) wherein location identifies which aptamer beacon is present. Aptamer beacon arrays are explained at col. 18, lines 44-67. Note that claim 17 is amended for clarity to include the definition of a distributed array as an array in which location does not identify the microcarrier.

In summary Stanton et al. does not teach or suggest the below features of claim 17:

- a hybridization assay for nucleic acid sequences or identifying said at least one nucleic acid sequence;
- a coding scheme comprising a plurality of signaling hairpins that are not target-binding probes (i.e., hybridizing probes in the language of claim 17);
- signaling hairpins (or other signaling moieties that are not probes for target molecules), some of which signal at a first level of an environmental condition such as temperature or denaturant concentration, and others of which signal only at a second level of that environmental condition;
- forming a distributed array of microcarriers, an array wherein location does not identify microcarriers having immobilized signaling hairpins; and
- optically decoding signaling hairpins (or any other signaling moieties) by changing a chemical or physical environmental condition to a first level, causing some to signal, and then to a second level, causing others to signal.

As explained below, the other references cited by the Examiner do not remedy the deficiencies of Stanton et al. noted above.

Liu et al.

While Liu et al. does teach hybridization probes for nucleic acid targets, Liu et al. does not remedy the other deficiencies noted in Stanton et al. above. As mentioned above, Liu et al. teaches immobilized probes that capture target to a solid surface and that are nucleic acid hybridization probes, namely, molecular beacon probes. Liu et al. does not teach the use of signaling hairpins for coding, nor does Liu et al. teach any other coding scheme. Contrary to the Examiner s assertion on page 9 of the Action, Liu et al. does not teach optically decoding the

fibers to identify a nucleic acid sequence as provided in claim 17. Rather, Liu et al. s fiber optic bundles are a standard positional array in that it is known by location which fiber, and thus which molecular beacon probe, has hybridized. Moreover, the distinction between claim 17 and Liu et al. has been made clear as claim 17 now recites that the decoding is accomplished by changing an environmental condition to detectably different levels to disrupt quenching and detecting changes in fluorescence from the signaling hairpins.

Lizardi et al.

Lizardi et al. does not teach any portion of what Stanton et al. and Liu et al. are lacking. Lizardi et al. teaches the use of separate capture probes so that non-specifically bound amplifiable hybridization probes can be washed away without losing the amplifiable hybridization probes that have hybridized to the target nucleic acid sequence (col. 9, lines 64-66). Lizardi et al. does not determine which hybridization probes have target hybridized to them, only that, if a positive result is obtained later in the assay ' after probes lead to a detectable amplification ' that some probes must have had target hybridized to them. Notably, Lizardi et al. does not teach any coding scheme. Contrary to the Examiner s assertions on page 11 of the action, Lizardi et al. does not provide motivation to utilize capture probes in the method of Stanton et al. Stanton et al. s aptamer beacons already perform the purpose of binding the non-nucleic acid targets to a solid surface and signaling, so there would appear to be no reason to use a separate capture probe as taught by Lizardi et al.

Chee et al.

Rather than supplying what Stanton et al. is missing, Chee et al. teaches away from the claimed invention. Chee et al. recognizes a problem with a prior bead-coding technique, namely, having to embed in each different microcarrier as it is being made a combination of signature molecules that can be detected to decode the microcarrier. Chee et al. s solution is different from the solution of the presently claimed invention. Chee et al. s solution is to carry out further rounds of hybridization to decode, not changing an environmental condition to decode. Chee et

al. immobilizes on the microcarriers, not signaling hairpins responsive to an environmental change, but rather identifier probes (IBL s) so that microcarriers whose hybridization probes have captured target can be contacted with labeled decoder probes (DBL s) to ascertain what combination of identifier probes each microcarrier carries. Chee et al. does not teach signaling hairpins, coding with signaling hairpins, or decoding by changing an environmental condition to see which hairpins signal at a first level of the environmental condition and which signal only at a second level. Chee et al. teaches that for a hybridization assay, the way to encode microcarriers is to utilize hybridization. Claim 17 recites a very different approach not suggested by Chee et al. At page 12 of the Action, the Examiner states that Chee et al. provides motivation to modify the fiber-optic method of Liu et al. to use encoded beads. Because Chee et al. teaches encoding beads by immobilizing thereon a combination of identifier probes to which complementary labeled decoder probes can be hybridized and detected in separate method steps, modifying Liu et al. in this fashion does not achieve the method of the claimed invention, nor does either Liu et al. or Chee et al. suggest the claimed method.

For the reasons stated above, the references cited by the Examiner do not teach or suggest each of the limitations recited in claim 17, and thus, the combination of references do not establish a *prima facie* case of obviousness. Because claims 18-22, 24-31, and 33-38 each depend, directly or indirectly, from claim 17, the cited references do not render these claim obvious for the same reasons discussed above. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

Claims 23 and 32

Claims 23 and 32 are rejected under 35 U.S.C. §103(a) as being unpatentable over Stanton et al. in view of Liu et al., Lizardi et al., and Chee et al. as applied to claims 17-22, 24-31, and 33-37 and further in view of Bonnet et al. (Proc. Natl. Acad. Sci., 96:6171-6176 (May 1999)).

Rejected claims 23 and 32 are dependent, either directly or indirectly, from independent claim 17. Since, as discussed above, the combination of Stanton et al., Liu et al., Lizardi et al., and Chee et al. does not teach or suggest all the elements/limitations of claim 17, even if Bonnet et al. discloses the additional limitations of applicants dependent claims 23 and 32 (arguendo), its combination with the other cited references still would not render obvious the claimed invention. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

Conclusion

In view of Applicant s claim amendments and the arguments presented above, the present application is believed to be in condition for allowance and an early notice thereof is earnestly solicited. If any issues remain, Applicants request that the Examiner contact the undersigned before issuing another action.

Respectfully submitted,

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